

diarrhea causing proliferative enteritis [1]. To the best of our knowledge, there is no report on *L. intracellularis* in the canine population in Korea. Therefore a serological and real-time PCR analysis were used to determine the prevalence of *L.intracellularis* in dogs with or without clinical signs of diarrhea.

Materials and Methods: Ninety two blood and fecal samples were obtained for serology and real-time PCR. Serum was separated by centrifugation at 3000 rpm for 15 minutes within 24 hours. Sera were analyzed by the commercial blocking enzyme linked immunosorbent assay (bELISA) kit (bioScreen, Germany) for the demonstration of specific antibodies against *L. intracellularis*. Positive and negative control sera were supplied by the manufacturer. The bELISA test on the serum samples was performed in the diagnostic laboratory, College of Veterinary Medicine, Chonbuk National University, Korea. Optical density was measured at 450 nm in a microplate reader and the percent inhibition (PI) of positive controls and test samples relative to the negative controls was calculated. Any serum sample with a PI of > 30% was considered positive. Any sample with a PI of < 20% was considered negative. Samples within the 20–30% range were considered suspicious. Fecal samples were processed for DNA purification according to the manufacturer's recommendations and were assayed for detection of *L. intracellularis* using real-time PCR assays. Statistically significant differences ($p < 0.05$) in serology and real-time PCR detection were determined by the Chi-square test using SPSS 17.0 (SPSS Inc., Chicago, IL, U.S.A.).

Results: Antibodies to *L. intracellularis* were observed 45 (48.91%) of 92 dogs tested and also 9 (9.78%) heads were suspected by bELISA. The higher prevalence (55.56%) was observed in < 5 years of aged dogs followed by 48.39%, 48.48% and 40% in 5-10 years, 11-15 years and >15 years respectively. Prevalence were also calculated as 56.52% in castrated males, 52.94% in spayed females, 44.44% in females and 44% in males. In breed, 12/21 in Maltese, 7/15 in Yorkshire Terrier, 2/10 in Shih Tzu, 3/12 in mixed, 5/7 in Cocker Spaniel, 2/4 in Jindo, 2/4 in poodle, 1/3 in Pekingese and 8/16 in others were antibody titer specific for *L. intracellularis*. In the group of GI disease ($n = 29$), 19 (65.17%) dogs were positive for *L. intracellularis*. Among positive dogs, 14 (73.68%) cases were diagnosed as enteritis and few of them with vomiting. In the group without GI disease ($n = 63$), 26 dogs were also positive (28.26%). Statistically, a significant ($p < 0.05$) differences were found between GI and non-GI diseased dogs. Furthermore, out of 92 fecal samples, 33 (35.87%) were identified as *L. intracellularis*-positive in real-time PCR assay.

Conclusions: In conclusion, despite the high prevalence of PPE in Korea, there is no published report on *L. intracellularis* infection in dogs in Korea. Since the presence of antibodies and Bacterial DNA indicate the exposure to bacterium and are not necessarily correlated to clinical disease. So, it is not possible to conclude whether the presence of antibodies in this dogs revealed disease or

was merely a sequel of a previous infection, subclinical infection or transient passage of the pathogen. Therefore, this high prevalence of *L. intracellularis* in dogs probably represents another important host species for this country.

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References

- [1] Lawson GH, Gebhart CJ. (2000). Proliferative enteropathy. J Comp Pathol 122:77-100.
- [2] Collins JE, Libal MC, Brost D. (1983). Proliferative enteritis in two pups. J Am Vet Med Assoc 183:886-889.
- [3] Leblanc B, Fox JG, Le Net JL, Masson MT, Picard A. (1993). Hyperplastic gastritis with intraepithelial Campylobacter-like organisms in a beagle dog. Vet Pathol 30:391-394.

P-077

Synergism of phage EcSw and antibiotics improved the effectiveness of the treatment against multi-drug resistant *E. coli* Sw1 isolated from pigs

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Introduction: Bacteriophages are a promising alternative to antibiotics for the reduction of pathogens. Therefore, continuing efforts are required to isolate novel phages with a broad host range to control or reduce the prevalence of antibiotic resistant *E. coli* strains. Naturally, most pathogenic strains are resistant to their specific phages. In addition, phage-antibiotic synergy has notable effects compared with the effects of phages or antibiotics alone. Moreover, minimizing the use of antibiotics is the recommended way to avoid the rapid emergence of antibiotic resistant bacteria.

Materials and Methods: Minimum inhibitory concentration (MIC) of antibiotics and Multiplicity of Infection (MOI) of phages was predicted by serial dilution and host cell lysis test as described previously (Easwaran, et al., 2015). Synergistic effect on the phage was examined by single-step growth curve and continuous culture assay. The titer of phage EcSw was estimated using double-agar-overlay assay.

Results: Combined therapy is less likely to fail due to resistance to one agent can kill by the second agent. In

here, the optimal MOI resultsshowed that MOI-1 had a significant inhibition rate than other concentration. Moreover, the results of MIC revealed that the higher concentration ofantibiotics can indirectly inhibit the titer of phage EcSw. The antibioticshave the ability to change the pattern of the phage life cycle in optimalconcentration were the results of single-step-growth curve. In combination withsub-lethal concentration of antibiotics, EcSw showed much higher levels ofantimicrobial activity than when phages or antibiotics were used alone. We alsoconfirmed that a combined treatment of antibiotics and phages can partiallyreduce the overuse of antibiotics.

Conclusions: This is useful to overcome thechallenges of antibiotic and phage resistance. Recently, the results confirmedthat the continuous culture experiments were more significantly control thegrowth of pathogenic strains.

References

- [1] Lu Z, Breidt Jr F, Fleming HP, Altermann E, Klaenhammer TR. Isolation and characterization of a Lactobacillus plantarum bacteriophage, phiJL-1, from a cucumber fermentation. *Int J Food Microbiol* 2003;84:225e35.
- [2] Goodridge L, Gallaccio A, Griffiths MW. Morphological, host range, and genetic characterization of two coli phages. *Appl Environ Microbiol* 2003;69:5364e71.

P-078

Diagnostic investigation of sudden death cases reported in swine farms and first isolation of *Clostridium Novyi* in Korea.

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Introduction: Sudden death cases without any significant lesion were often reported in finishing pigs and sows during the summer and it causes significant economic losses to individual swine farms. Generally, sudden death in finishing pigs or sows was caused by various factors such as bacteria and/or virus infection, nutrition, physical and environmental factors. Especially during the summer when temperature and humidity are extremely high, the number of sudden death case in swine farms is drastically increased. In the study, sudden death cases reported in pig farms were investigated to determine the possible reasons for the sudden death.

Materials and Methods: Total of 12 cases of sudden death were submitted to Chonbuk National University Diagnostic Center (CBNU-VDC) between June and September, 2015. All of the submitted cases were subjected to necropsy, then various diagnostic tests such as PCR, ELISA, bacterial culture, virus isolation, and histopathology to identify possible causative pathogens.

Results: Identified pathogens detected from the submitted cases were *Clostridium novyi* type A(1 case) and type B

(3 cases), *Clostridium perfringens* (1 case) and *Salmonella* Typhimurium(2 cases) for bacterial agents. In addition, PRRSV (1 case) and PCV2 (1 case) were identified for viral agents from the cases. No pathogen was detected from 5 cases.

Conclusions: Sudden death cases without any significant lesion were often reported in finishing pigs and sows during the summer and total of 12 cases of sudden death were submitted to CBNU-VDC during the summer 2015. *Clostridium novyi* was isolated from 4 cases and these are the first field isolates reported in Korea. *Clostridium perfringens* and *Salmonella* Typhimurium were also isolated from 3 cases, indicating that bacterial pathogens might be the main causative agents for sudden death of finishing pigs or sows during the summer season.

References

- [1] Brazier JS, Duerden BI, Hall V, Salmon JE, Hood J, Brett MM, McLauchlin J, George RC: Isolation and identification of Clostridium spp. from infections associated with the injection of drugs: experiences of amicrobiological investigation team. *J Med Microbiol* 2002, 51(11):985-989.
- [2] Wilson MR: Sudden death in pigs. *Can Vet J* 1970, 11(9):178-180.
- [3] Sasaki Y, Kojima A, Aoki H, Ogikubo Y, Takikawa N, Tamura Y: Phylogenetic analysis and PCR detection of Clostridium chauvoei, Clostridium haemolyticum, Clostridium novyi types A and B, and Clostridium septicum based on the flagellin gene. *Vet Microbiol* 2002, 86(3):257-267.

P-079

The effects of bacteriophage treatment in pigs challenge with an edema-diseases-causing strain of *E. coli*

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Introduction: Edema disease (ED) is caused pig and wild animals by Shiga toxin producing *E. coli* (STEC). Also, STEC causes a severe illness including hemorrhagic colitis and hemolytic-uremic syndrome in humans. Damage to the vascular endothelium results in edema, hemorrhage, and microthrombosis and ultimately results in high mortality in STEC-infected pigs. The new law that prohibits the use of antibiotics in animal feeds in Korea, development of antimicrobial alternatives became necessary. Among various alternatives, bacteriophages had received great attention as a possible alternative for antibiotics in livestock industry because it has been demonstrated that bacteriophages are non-hazardous self-replicating agent that can infect and multiply in bacteria to prevent bacterial diseases in previous studies. In this study, we confirmed that STEC growth was inhibited by the specific *E. coli* bacteriophage and investigated the pig intestinal bacterial flora after *E. coli*